JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 1991, p. 2120–2124
0095-1137/91/102120-05$02.00/0

Vol. 29, No. 10

Identification of *Toxoplasma gondii* Infections by BI Gene Amplification

E. VAN DE VEN,* W. MELCHERS, J. GALAMA, W. CAMPS, AND J. MEUWISSEN

Department of Medical Microbiology, University of Nijmegen, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands

Received 25 March 1991/Accepted 9 July 1991

The diagnosis of toxoplasmosis in congenitally infected children or in immunocompromised patients can be difficult; serology is not reliable, and the diagnosis must be based on the combination of symptomatology and the direct demonstration of the parasite in clinical specimens by microscopy, antigen detection, or inoculation of samples into mice or tissue cultures. These techniques are either insensitive or time-consuming. To determine the value of the polymerase chain reaction (PCR) for the diagnosis of *Toxoplasma gondii* infections, we compared this technique with conventional detection techniques, such as microscopy, tissue culturing, and mouse inoculation. We were able to detect *T. gondii* by PCR in clinical specimens and tissue samples that were obtained postmortem from a bone marrow recipient with cerebral toxoplasmosis and from three congenitally infected children. The presence of *T. gondii* was demonstrated in brain tissue, cerebrospinal fluid, the heart, and skeletal muscle tested fresh or after fixation in Formalin. In only one sample was *T. gondii* isolated by mouse inoculation but not detected by PCR. Because it is a sensitive, relatively rapid, and specific method and because it can be applied to a variety of different clinical samples, PCR can be considered a valuable additional tool for the identification of *T. gondii* infections.

Toxoplasmosis is an infectious disease caused by the protozoan *Toxoplasma gondii*. In healthy individuals, the course of toxoplasmosis is harmless and frequently without symptoms. However, in some individuals, such as congenitally infected children (7, 20) and immunocompromised patients (AIDS patients [17, 18] and transplant recipients [6, 11, 19]), toxoplasmosis can be life-threatening. Congenital toxoplasmosis can cause fetal death or severe neurological sequelae, such as hydrocephalus, microcephalus, or blindness (20). The most common manifestation of a recurrent *T. gondii* infection in immunocompromised patients, especially AIDS patients, is focal encephalitis (17, 18). The extent of damage can be reduced by early treatment, for which a rapid diagnosis is mandatory. The diagnosis is routinely based on serological tests. In this category of patients, however, serology is inadequate because antibody production either fails or is significantly delayed (8, 11, 13, 17). Moreover, the demonstration of antibodies in neonates is hampered by the presence of maternal immunoglobulin G (IgG) (13). Therefore, direct methods to demonstrate the presence of the parasite in tissues or body fluids are more useful. Of these direct methods, light microscopy is insensitive and antigen detection is unreliable (11, 17–19). Tissue culturing and mouse inoculation are specific and sensitive but time-consuming; it can take up to 6 weeks to obtain a diagnosis (8).

Polymerase chain reaction (PCR) assays which make use of primers selected either from the P30 or the BI gene of *T. gondii* have been described (4, 5). A sensitivity of 10 genome equivalents in the presence of 10^5 human leucocytes has been reported (4), but so far only a few studies have reported on the clinical application of PCR to toxoplasmosis (10, 14).

In this paper, we present a comparison of PCR with conventional diagnostic methods for the diagnosis of toxoplasmosis. A variety of clinical samples were tested fresh, after storage at −80°C, or after fixation in Formalin.

* Corresponding author.

MATERIALS AND METHODS

(i) Case report 1. A 32-year-old man with acute myelogenous leukemia underwent a transplantation with allogeneic bone marrow. Shortly after transplantation, he developed grade II graft-versus-host disease of the skin. Treatment with corticosteroids was partially effective; therefore, prolonged immunosuppression with a combination therapy of steroids and azathioprine was given, resulting in stabilization of his chronic graft-versus-host disease. Seven months after bone marrow transplantation, the patient suffered from progressive headaches and left-sided hemiplegia. A computerized tomographic scan of the brain revealed lesions in the right hemisphere. Because antibiotic treatment for suspected brain abscesses was not effective and the condition of the patient deteriorated, a biopsy specimen was taken from the brain. The patient, who was seropositive for toxoplasmosis before transplantation, no longer had antibodies against *T. gondii* detectable at the time of the brain biopsy. Despite therapy with pyrimethamine and sulfadiazine, the patient died 7 days after the brain biopsy. At autopsy, specimens of brain, lung, lymph nodes, heart, bone marrow, spleen, kidney, adrenal gland, skeletal muscle, and liver tissues were obtained to test for the presence of *T. gondii*.

(ii) Case report 2. At 36 weeks of gestation, a child suffering from hydrocephalus and chorioretinitis was born. During pregnancy, abnormalities of the fetal skull were noted by ultrasomography. The mother had no symptoms suggestive of a *T. gondii* infection; therefore, she had not been tested serologically for toxoplasmosis. A computerized tomographic scan revealed enlargements of the third and lateral ventricles of the child's brain. The diagnosis was based on the demonstration of the intrathecal production of IgG antibody against *T. gondii* and the direct demonstration of the parasite. IgM antibody was not detected. The patient was treated for 21 days with pyrimethamine and sulfadiazine and for 40 days with spiramycin. Cerebrospinal fluid (CSF) was obtained 40 and 81 days after birth and tested both for

the presence of *T. gondii* directly and the presence of antibody.

(iii) Case report 3. After 12 weeks of pregnancy, a woman became ill with fever and malaise. She had an enlarged cervical lymph node, but this was not investigated. Three weeks later, growth retardation of the fetus was diagnosed. Serological screening of the woman for antibody against *T. gondii* was negative early in pregnancy, but at 16 weeks both IgM and IgG antibodies to *T. gondii* were present. At 17 weeks of pregnancy, the woman was admitted to another hospital, where fetal death was diagnosed. The woman had not been treated for toxoplasmosis. Delivery was induced with prostaglandins, and a fetus of about 17 weeks was delivered. It was transferred to our laboratory for examination. The brain, lungs, liver, and umbilical cord were examined for the presence of *T. gondii*.

(iv) Case report 4. A 35-year-old woman had an unnoticed *T. gondii* infection during pregnancy. At 33 weeks of gestation, hydrocephalus of the fetus was diagnosed by ultrasomography.One week later, fetal death occurred and delivery took place. Hydrocephalus was confirmed at autopsy of the fetus. Histological examination revealed *T. gondii* in the brain. Formalin-fixed tissues were send to our laboratory for confirmation.

Preparation of the samples. Clinical tissues were cut and homogenized in lysis buffer (100 mM NaCl, 10 mM Tris-HCl [pH 8.0], 25 mM EDTA, and 0.5% sodium dodecyl sulfate [SDS] with 0.1 mg of proteinase K [1] [Promega, Leiden, The Netherlands] per ml). CSF and Formalin-fixed tissues were treated with proteinase K in 0.5% SDS. Incubation took place for 12 to 18 h at 50°C and was followed by extraction with phenol, phenol-chloroform-isoamyl alcohol (25:24:1), and chloroform-isoamyl alcohol (24:1) successively. The DNA was precipitated with ethanol overnight at −20°C (1). The concentration of isolated human DNA was estimated on a 1% agarose gel, and amounts between 0.1 and 1.0 μg were examined for the presence of *T. gondii* DNA by PCR.

PCR. Amplification of *T. gondii* DNA was carried out in a 100-μl reaction mixture containing 50 mM KCl; 10 mM Tris-HCl (pH 8.3); 2.5 mM $MgCl_2$; 0.01% gelatin; 200 μM each dATP, dGTP, dCTP, and dTTP; 100 pmol of each primer; and 2 U of *Taq* DNA polymerase (Perkin-Elmer Cetus). *T. gondii* primers 1 and 4, described by Burg et al. (4), were modified by 1 nucleotide to avoid primer-dimer amplification. The new primers were 5′-TTGCATAGGTTG CAGTCACT-3′ (positions 694 to 714) (primer 1) and 5′-TCTTTAAAGCGTTCGTGGTC-3′ (positions 868 to 888) (primer 4). The primers are located on the BI gene. This gene is about 35-fold repetitive, and its function is unknown. The reaction mixture was overlaid with 2 drops of mineral oil to prevent evaporation, and 55 cycles of amplification were performed in a PCR Thermocycler (Perkin-Elmer Cetus). Each cycle consisted of 1 min of denaturation at 94°C, 30 s at the annealing temperature of 42°C, and 2 min of extension at 72°C. For prevention of contamination, strict spatial partitioning of the different technical steps of PCR, the use of pipets with disposable pistons, and frequent changes of gloves were included (16).

Southern blotting. After the last PCR cycle, 30 μl of the reaction mixture was analyzed by electrophoresis on a 2% agarose gel (1). For Southern blotting, the agarose gel was depurinated in 0.25 N HCl and transferred to a nylon membrane (Hybond; Amersham) by diffusion blotting in 0.4 N NaOH. The DNA was covalently bound to the nylon membrane by UV irradiation on a UV transilluminator at 254 nm for 5 min.

Dot spotting. For dot spotting, 30 μl of the amplified reaction mixture was denatured with 7.4% formaldehyde in 5× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) at 65°C for 10 min. The DNA was spotted on a nylon membrane with a dot spot apparatus (Bethesda Research Laboratories), and the DNA was covalently bound to the nylon membrane as described for Southern blotting.

Hybridization assay. The specificity of the amplified product was confirmed by hybridization with oligonucleotide 3 (5′-GGCGACCAATCTGCGAATACACC-3′ [positions 831 to 853]), a sequence within the region being amplified (4). The filter was prehybridized in 6× SSC–5× Denhardt solution (100× Denhardt solution is 2% bovine serum albumin, 2% Ficoll, and 2% polyvinylpyrrolidone)–0.1% SDS–250 μg of salmon sperm DNA per ml at 42°C for 1 h. Hybridization took place in 6× SSC–1× Denhardt solution–0.1% SDS–250 μg of salmon sperm DNA per ml at 42°C for 15 h with ^{32}P-end-labeled oligonucleotide 3 (specific activity, >5,000 Ci/mmol). After hybridization, the filter was washed twice in 2× SSC–0.1% SDS at 55°C for 15 min each time and once in 0.5× SSC–0.1% SDS at 55°C for 15 min. The blots were autoradiographed for 4 h on Kodak Royal X-Omat film between intensifying screens at −80°C.

Mouse inoculation. Fresh tissues were homogenized in saline in a mortar, and 400 IU of penicillin and 0.1 mg of gentamicin per ml were added. Two or three male Swiss Webster mice were inoculated with 0.2 ml of the suspension subcutaneously and 0.5 to 1.0 ml intraperitoneally. CSF and buffy coat cells (0.02 ml each) were inoculated intracerebrally. Inoculated mice were monitored for up to 45 days. Animals that died during this period were examined for the presence of *T. gondii* in peritoneal exudates or in the brain. Surviving mice were sacrificed at 45 days, and serum samples taken before inoculation and at 45 days were tested serologically by the immunofluorescent-antibody test. Histological examination of peritoneal exudates and the brain was performed as well.

Immunofluorescence assay. Indirect immunofluorescence for the detection of *T. gondii* antibodies in mouse sera was performed on infected chicken brain sections with fluorescein-labeled anti-IgG globulins as described by van Nunen and van der Veen (25). Mouse sera were tested at a dilution of 1:20.

Tissue culturing. Tissues were homogenized as described above for mouse inoculation. The suspension was pushed through a 27-gauge needle, and 1 ml was seeded on HEp-2 cell cultures (25 cm^2). The cells were maintained in Eagle's minimal essential medium supplemented with nonessential amino acids, 3% heat-inactivated fetal bovine serum, 20 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES), penicillin (100 IU/ml), and gentamicin (0.5 mg/ml). The medium was changed after 1, 3, 6, 9, and 12 days. If the HEp-2 monolayers were damaged, the suspension was transferred to fresh HEp-2 monolayers. Identification of *T. gondii* in cell cultures was done by examination with an inverted microscope, directly or by the immunofluorescent-antibody test.

Serology. IgM and IgG class antibodies against *T. gondii* were detected by means of direct and indirect enzyme-linked immunosorbent assays, which have been described before (23, 24).

Microscopy. Fresh materials were frozen directly in liquid nitrogen. Sections (5 μm) of the samples were cut in a cryostat at −20°C and fixed in acetone at −20°C. CSF was

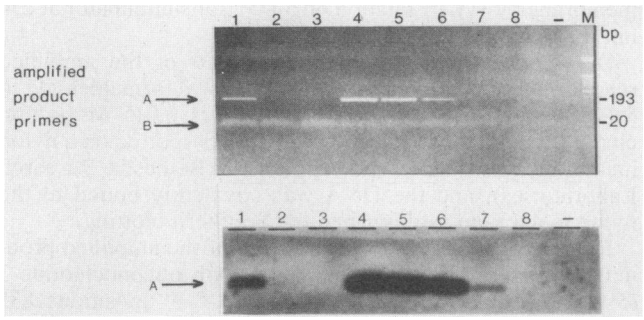

FIG. 1. Gel electrophoresis and Southern blot hybridization of the DNA obtained by brain biopsy in case report 1 and tested by PCR. Three different amounts of DNA were tested for the presence of *T. gondii* by PCR. Lanes: 1, 100 ng of brain tissue DNA; 2, 250 ng of brain tissue DNA; 3, 1 μg of brain tissue DNA; 4 to 8, concentration range of *T. gondii* DNA (15 ng, 0.15 ng, 1.5 pg, 15 fg, and 0.15 fg, respectively); –, distilled water (negative control); M, size markers (pBR322 digested with *Hin*fI); A, amplified *T. gondii*-specific product of 193 bp; B, primers (20 bp).

attached to a poly-L-lysine-coated slide by centrifugation and subsequently fixed with methanol. All sections were stained by the Giemsa method and examined under the microscope.

RESULTS

The brain biopsy from a bone marrow recipient (case report 1), positive for *T. gondii* by light microscopy, also was tested by PCR. PCR resulted in specific amplification when 0.1 μg of sample DNA was tested but not when 0.25 or 1.0 μg of sample DNA was tested. The specificity was confirmed by Southern blot hybridization with the internal oligonucleotide probe (Fig. 1).

After death, autopsy samples from various tissues were obtained and tested for the presence of *T. gondii*, both by conventional methods and by PCR. The results are summarized in Table 1. To check for cross-contamination during sample preparation, we included a lymph node and a spleen from two patients serologically negative for toxoplasmosis as controls.

T. gondii DNA could be detected by PCR in the skeletal muscle, brain, and heart tissues from the bone marrow recipient directly on a gel (Fig. 2). Positivity was confirmed by dot spot hybridization (Fig. 3). The other samples, including the negative lymph node and spleen obtained from *T. gondii*-negative patients, remained negative by PCR. These results were in agreement with those of *T. gondii* isolation procedures and microscopic examination (Table 1). However, the lymph node biopsy from this patient was found positive by mouse inoculation but negative by PCR.

T. gondii was also detected in the CSF (case report 2) by all techniques used, including PCR (Table 1). After the CSF, taken 8 days after birth, was found positive for *T. gondii*, therapy was started, and the CSF was reexamined for *T. gondii* after 40 and 81 days. Despite therapy, parasites were still present in the CSF at day 40, as demonstrated by all techniques (Table 1). At day 81, *T. gondii* could no longer be detected in the CSF. CSF from a seronegative patient without suspicion of toxoplasmosis served as a negative control.

The brain of the fetus that died in utero (case report 3) was found positive for *T. gondii* by both PCR and mouse inoculation but not by tissue culturing (Table 1). A stronger signal was observed when double the amount of sample DNA was used (Fig. 4). The liver, lungs, and umbilical cord were negative (Table 1).

Formalin-fixed tissues from a fetus that died in utero because of congenital toxoplasmosis (case report 4) were tested to investigate whether PCR is also useful for fixed

TABLE 1. *T. gondii* detection in clinical specimens

Case report and specimen[a]	Result[b] determined by:			
	Microscopy	Tissue culturing	Mouse inoculation (IFT)[c]	PCR
1				
Brain biopsy	+	ND	ND	+
Brain	+	–	+	+
Skeletal muscle	+	+	+	+
Heart	+	+	+	+
Spleen	–	–	–	–
Adrenal gland	–	–	–	–
Kidney	–	–	–	–
Liver	–	–	–	–
Lymph node	–	–	+	–
Lung	–	–	–	–
Bone marrow	–	–	–	–
CSF	–	ND	–	–
2, CSF				
Day 8	+	+	+	+
Day 40	+	+	+	+
Day 81	–	–	–	–
3				
Brain	–	–	+	+
Liver	–	–	–	–
Lung	–	–	–	–
Umbilical cord	–	–	–	–
4				
Brain	+	ND	ND	+
Heart	–	ND	ND	–
Liver	–	ND	ND	–
Spleen	–	ND	ND	–
Kidney	–	ND	ND	–

[a] For case reports 1, 3, and 4, all specimens, except for brain biopsy of case report 1, were obtained at autopsy and Formalin fixed.
[b] +, positive; –, negative; ND, not done.
[c] IFT, immunofluorescent antibody test.

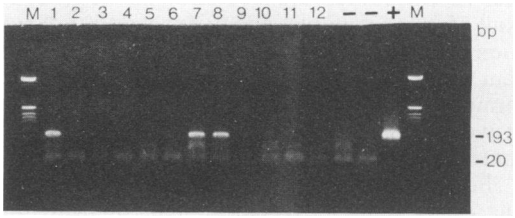

FIG. 2. PCR of biopsies taken at autopsy from a bone marrow transplantation patient (case report 1) analyzed by gel electrophoresis. For each biopsy, the optimal DNA concentration was established by PCR with several dilutions. Lanes: 1, skeletal muscle; 2, adrenal gland; 3, liver; 4, lymph node; 5, bone marrow; 6, lung; 7, brain; 8, heart; 9, kidney; 10, spleen; 11, *T. gondii*-negative lymph node; 12, *T. gondii*-negative spleen; –, distilled water (negative control); +, 15 ng of *T. gondii* DNA (positive control); M, size markers (pBR322 digested with *Hin*fI).

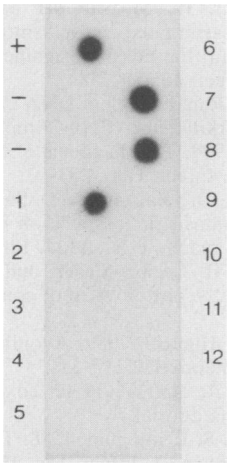

FIG. 3. Hybridization of the PCR products shown in Fig. 2 with the internal probe in a dot spot assay. The numbers of the samples correspond to the numbers in Fig. 2.

materials. It was possible to detect *T. gondii* in Formalin-fixed brain tissue by PCR after dot spot hybridization. The heart, liver, spleen, and kidneys remained negative after amplification (Fig. 5). These results were confirmed by microscopic examination (Table 1).

DISCUSSION

A primary *T. gondii* infection during pregnancy leads to transmission and infection of the fetus in only one-third of cases (7). Therefore, an antenatal diagnosis of congenital infection is very helpful in making an informed decision on either treatment or therapeutic intervention. Serology is not reliable, but parasites have successfully been isolated from amnionic fluid by mouse inoculation or tissue culturing (10). Congenitally infected children, apparently healthy at birth, can develop ocular toxoplasmosis later in life, even after 20 years (15). PCR can be very helpful in cases of atypical retinitis or when the fundus is masked by vitreal inflammation. Recently, Brézin and coworkers demonstrated the analysis of ocular toxoplasmosis in the aqueous humor by PCR (3).

Similar problems exist in the case of immunodeficiency, in which serology cannot be used because toxoplasmosis is mainly caused by endogenous reactivation and IgG antibodies will be present from the beginning on. Actually, the titers may decrease during active infection and the IgM response may fail (11). Therapeutic treatment of immunocompromised patients mostly relies on a high index of suspicion instead of a confirmed diagnosis (17).

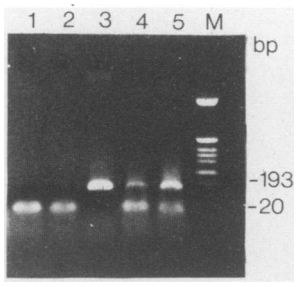

FIG. 4. PCR of DNA isolated from the brain tissue from a fetus that died in utero (case report 3). Estimated DNA amounts used for amplification were 150 ng (lane 4) and 300 ng (lane 5). Two negative controls containing distilled water (lanes 1 and 2) and a positive control containing 15 ng of *T. gondii* DNA (lane 3) were included. M, size markers (pBR322 digested with *Hin*fII).

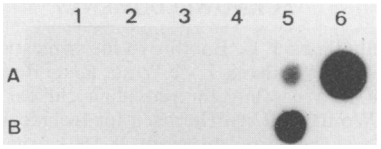

FIG. 5. Dot spot hybridization after amplification of DNA isolated from Formalin-fixed tissues from a congenitally infected infant (case report 4). The DNA was totally degraded after isolation; DNA concentrations were estimated to be 0.1 μg (row A) and 0.5 μg (row B). Lanes: 1, heart; 2, liver; 3, kidney; 4, spleen; 5, brain; 6, distilled water (B) and 15 ng of *T. gondii* DNA (A).

Therefore, a rapid diagnostic technique was sought with PCR. On tissue sections, however, PCR cannot discriminate between a latent (cyst-stage) infection and an active infection. This problem may be circumvented by testing on blood; Shepp et al. (21) found parasitemia due to reactivation in three bone marrow recipients. Blood may be also the site of preference to sample for an antenatal diagnosis of toxoplasmosis. Desmonts et al. isolated *T. gondii* from fetal blood obtained by cordocentesis (8). The detection level of PCR is 10 parasites in the presence of 10^5 human leucocytes (4). However, the sensitivity of PCR in clinical samples may be influenced by the presence of inhibitory factors, such as porphyrins, which can inhibit the reaction in blood samples (12). Unknown inhibitory components, comparable to porphyrins in blood, may explain the negative PCR result in case report 1, in which amplification of 0.25 and 1.0 μg of template DNA gave a negative result, while that of 0.1 μg gave a positive result (Fig. 1). The presence of extra human DNA probably does not hinder primer annealing. In case report 3, a stronger signal was observed when double the amount of sample DNA was used. Inhibitory components may also explain the negative PCR result for the lymph node (case report 1), from which the parasite could be isolated in mice.

In this study, we showed that it is possible to detect parasites by PCR in a variety of different clinical specimens, including Formalin-fixed tissue. The latter is an advantage when the material must be stored or transported. Goelz et al. (9) showed that DNA isolation of Formalin-fixed material was possible, although the DNA was degraded. Recently, Tokuda et al. (22) found that this degraded DNA could be used for PCR, and our results confirm this finding.

As soon as PCR is reliable for the detection of *T. gondii* in blood specimens, it should be added to the repertoire for the diagnosis of active *T. gondii* infections. We are currently working on antenatal detection of *T. gondii* in amniotic fluid and fetal blood by PCR in a rhesus monkey model. Preliminary results for blood samples with PCR and a DNA isolation method described by Boom et al. (2) are promising. In conclusion, PCR can be used as an additional diagnostic tool for the rapid detection of *T. gondii* in various clinical materials. PCR may provide a diagnosis within 3 days of receiving a sample. It is expected that PCR for the detection of this parasite in blood samples will be developed in the near future. Such a development will increase the value of PCR as a tool for the diagnosis of active toxoplasmosis.

ACKNOWLEDGMENTS

We gratefully thank J. C. Boothroyd for suggestions in the initial stage of this work. We thank T. de Witte, R. de Boer, W. Kwee, R. Mullaart, and P. Wesseling for providing clinical specimens and information. We thank Theo Hermsen for technical assistance.

This work was supported by grant 28-1604 from the Prevention Fund of The Netherlands.

REFERENCES

1. **Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl.** 1989. Current protocols in molecular biology. John Wiley & Sons, Inc., New York.
2. **Boom, R., C. J. A. Sol, M. M. M. Salimans, C. L. Jansen, P. M. E. Wertheim-van Dillen, and J. van der Noordaa.** 1990. Rapid and simple method for purification of nucleic acids. J. Clin. Microbiol. **28**:495–503.
3. **Brézin, A. P., C. Silveira, P. Thulliez, M. C. Martins, R. M. Mahdi, R. Belfort, and R. B. Nussenblatt.** 1991. Analysis of aqueous humor in ocular toxoplasmosis. N. Engl. J. Med. **324**:699.
4. **Burg, J. L., C. M. Grover, P. Pouletty, and J. C. Boothroyd.** 1989. Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii*, by polymerase chain reaction. J. Clin. Microbiol. **27**:1787–1792.
5. **Burg, J. L., D. Perelman, L. H. Kasper, P. L. Ware, and J. C. Boothroyd.** 1988. Molecular analysis of the gene encoding the major surface antigen of *Toxoplasma gondii*. J. Immunol. **141**: 3584–3591.
6. **Derouin, F., E. Gluckman, B. Beauvais, A. Devergie, R. Melo, M. Monny, and M. Lariviere.** 1986. Toxoplasma infection after human allogeneic bone marrow transplantation: clinical and serological study of 80 patients. Bone Marrow Transplant. **1**:67–73.
7. **Desmonts, G., and J. Couvreur.** 1974. Congenital toxoplasmosis: a prospective study of 378 pregnancies. N. Engl. J. Med. **290**:1110–1116.
8. **Desmonts, G., F. Forestier, P. Thulliez, F. Daffos, M. Capella-Pavlovsky, and M. Chartier.** 1985. Prenatal diagnosis of congenital toxoplasmosis. Lancet **i**:500–504.
9. **Goelz, S. E., S. R. Hamilton, and B. Vogelstein.** 1985. Purification of DNA from formaldehyde fixed and paraffin embedded human tissue. Biochem. Biophys. Res. Commun. **130**:118–126.
10. **Grover, C. M., P. Thulliez, J. S. Remington, and J. C. Boothroyd.** 1990. Rapid prenatal diagnosis of congenital *Toxoplasma* infection by using polymerase chain reaction and amniotic fluid. J. Clin. Microbiol. **28**:2297–2301.
11. **Heurkens, A. H. M., I. A. Koelma, M. M. de Planque, A. M. Polderman, and J. W. M. van der Meer.** 1989. Failure to diagnose fatal disseminated toxoplasmosis in a bone marrow transplant recipient: the possible significance of declining antibody titers. J. Infect. **18**:283–288.
12. **Higuchi, R.** 1989. Rapid, efficient DNA extraction for PCR from cells or blood. Perkin Elmer Cetus Amplifications **2**:1–3.
13. **Holliman, R. E.** 1990. The diagnosis of toxoplasmosis. Serodiagn. Immunother. Infect. Dis. **4**:83–93.
14. **Holliman, R. E., J. D. Johnson, and D. Savva.** 1990. Diagnosis of cerebral toxoplasmosis in association with AIDS using the polymerase chain reaction. Scand. J. Infect. Dis. **22**:243–244.
15. **Koppe, J. G., D. H. Loewer-Sieger, and H. de Roever-Bonnet.** 1986. Results of 20-year follow-up of congenital toxoplasmosis. Lancet **ii**:254–255.
16. **Kwok, S., and R. Higuchi.** 1989. Avoiding false positives with PCR. Nature (London) **339**:237–238.
17. **Luft, B. J., and R. Hafner.** 1990. Toxoplasmic encephalitis. AIDS **4**:593–595. (Editorial.)
18. **Luft, B. J., and J. S. Remington.** 1988. Toxoplasmic encephalitis. AIDS commentary. J. Infect. Dis. **157**:1–6.
19. **Pendry, K., R. C. Tait, A. McLay, D. Ho Yen, D. Baird, and A. K. Burnett.** 1990. Toxoplasmosis after BMT for CML. Bone Marrow Transplant. **5**:65–67.
20. **Rahman, M. S., and J. Rahman.** 1985. Toxoplasma in pregnancy, p. 45–55. *In* L. G. Keith, G. S. Berger, and D. A. Edelman (ed.), Infections in reproductive health, vol. II. Uncommon infections and special topics. MTP Press Ltd., Falcon House, Lancaster, England.
21. **Shepp, D. H., R. C. Hackman, F. K. Conley, J. B. Anderson, and J. D. Meyers.** 1985. *Toxoplasma gondii* reactivation identified by detection of parasitemia in tissue culture. Ann. Intern. Med. **103**:218–221.
22. **Tokuda, Y., T. Nakamura, K. Satonaka, S. Maeda, K. Doi, S. Baba, and T. Sugiyama.** 1990. Fundamental study on the mechanism of DNA degradation in tissues fixed in formaldehyde. J. Clin. Pathol. **43**:748–751.
23. **van Loon, A. M., J. T. M. van der Logt, F. W. A. Heessen, and J. van der Veen.** 1983. Enzyme-linked immunosorbent assay that uses labeled antigen for detection of immunoglobulin M and A antibodies in toxoplasmosis: comparison with indirect immunofluorescence and double-sandwich enzyme-linked immunosorbent assay. J. Clin. Microbiol. **17**:997–1004.
24. **van Loon, A. M., and J. van der Veen.** 1980. Enzyme-linked immunosorbent assay for quantitation of Toxoplasma antibodies in human sera. J. Clin. Pathol. **33**:635–639.
25. **van Nunen, M. C. J., and J. van der Veen.** 1965. Examination for toxoplasmosis by fluorescent antibody technique. Trop. Geogr. Med. **17**:246–253.